Factors Affecting the Infectivity of Lymphocytes from Cattle with Bovine Leukosis Virus

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ABSTRACT

Peripheral blood mononuclear cells were obtained from 13 bovine leukosis virus infected cattle and inoculated subcutaneously into 29 recipient adult steers to determine (a) the number of mononuclear cells (equivalent amount of blood) necessary to cause infection and (b) factors influencing infectivity of mononuclear cells from bovine leukosis virus-infected animals. A total of 55 inoculations were made. Inoculation of 1×10^4, 2×10^4 and 5×10^4 mononuclear cells caused seroconversion in 12%, 57% and 62% of steers, respectively. No infections occurred with 1×10^3 or 2×10^3 mononuclear cells. Cattle infected for longer than 24 months and those animals greater than three years of age were more likely to cause infection with 1 to 5×10^4 mononuclear cells than were cattle infected for less than 24 months or animals less than three years of age. Lymphocytes from cattle with persistent lymphocytosis caused more infections when 1×10^4 or 2×10^4 mononuclear cells were inoculated, than did lymphocytes from nonpersistent lymphocytosis cattle; however, both groups were equally infectious when 5×10^4 mononuclear cells were inoculated. No differences were found in infectivity of experimentally vs naturally exposed animals.

Key Words: Bovine leukosis virus, lymphoma, persistent lymphocytosis, lymphocytes, cattle.

RÉSUMÉ

Cette expérience consistait à récolter des mononucléaires du sang périphérique, chez 13 bovins infectés par le virus de la leucose bovine, et à en effectuer 55 inoculations dans le tissu sous-cutané de 29 bouvillons adultes, afin de déterminer le nombre de mononucléaires, ou la quantité équivalente de sang, nécessaire pour causer l'infection, ainsi que les facteurs qui influencent l'infectivité des mononucléaires des bovins atteints de leucose. L'injection de 1×10^4, 2×10^4 et 5×10^4 mononucléaires provoqua une réaction sérologique, chez respectivement 12%, 57% et 62% des bouvillons, contrairement à l'injection de seulement 1×10^3 ou 2×10^3 de tels mononucléaires. L'injection de 1 à 5×10^4 mononucléaires récoltés chez des sujets infectés depuis plus de 24 mois, ou âgés de plus de trois ans, se révéla plus susceptible de causer l'infection, comparativement à l'injection de la même quantité de mononucléaires qui provenaient de sujets infectés depuis moins de 24 mois, ou âgés de moins de trois ans. Le lymphocytes des bovins qui affichaient une lymphocytose persistante causèrent plus de cas d'infection, à la suite de l'injection de 1×10^4 ou 2×10^4 mononucléaires, que le firent ceux des bovins qui n'affichaient pas une telle lymphocytose; les lymphocytes de ces deux groupes de bovins s'avérèrent toutefois également infectieux, lorsque l'inoculum contenait 5×10^4 mononucléaires. Le fait que les bovins soient atteints de leucose naturelle ou expérimentale n'entraîna pas de différence décelable.

Mots clés: virus de la leucose bovine, lymphome, lymphocytose persistante, lymphocytes, bovins.

INTRODUCTION

Bovine leukosis virus (BLV), presumed to be the causative agent of the adult form of bovine lymphosarcoma, infects approximately 20% of dairy cattle in the United States (1). Infection rates as high as 42% and 48% have been reported for dairy cattle in Alabama (R.D. Schultz, personal communication) and Florida (2), respectively. Ferrer (1) estimates that less than 5% of cattle infected with BLV develop lymphoma whereas, Schultz estimates that only 0.1% of infected cattle become clinically diseased (personal communication). Persistent lymphocytosis (PL), a benign lymphoproliferative condition characteristic of many chronic diseases of cattle, accompanies approximately 29% of BLV infections (3). Cattle with PL are clinically normal; neither milk production nor reproduction are affected by the condition (4,5). The susceptibility to both lymphosarcoma and PL is genetically controlled, occurring more commonly in certain cattle families (6,7). A separate genetic control is postulated because cattle families characteristically developing PL may not develop lymphosarcoma and vice versa (6,7).

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Bovine leukosis virus replicates in lymphocytes (8,9,10), being detectable by electron microscopy in peripheral blood lymphocytes or milk lymphocytes after *in vitro* cultivation (11,12,13). The virus is highly cell-associated; cell-free virus has rarely been reported (8). Serological evidence suggests that horizontal transmission is the major route of spread of BLV (14,15). The transfer of infectious lymphocytes (e.g. blood) among animals is thought to be essential for horizontal transmission. For this reason, blood sucking insects have been suggested to play an important role as vectors of BLV. The transmission of BLV to sheep via the bite of tabanids that had fed on an infected cow (16), and by subcutaneous inoculation of mouthparts from mosquitoes fed on BLV-infected blood (17) supports this suggestion.

The importance of insects as vectors depends on the quantity of blood from a BLV-infected animal required to cause infection. Van Der Maaten and Miller (18) reported that 2500 lymphocytes (5 x 10^{-4} mL of blood) caused infection in a calf. This type of study has never been extended to determine the number of lymphocytes from BLV-infected cattle that constitute an infectious dose for adult cattle. Little information is available on factors affecting the infectivity of BLV-infected cattle. The purpose of the present study was to determine (a) the number of lymphocytes (quantity of blood) from BLV-infected cattle causing infection in adult cattle and (b) the effect of experimental vs natural infection of the donor and PL, duration of infection and age of the donor on the infectivity of its lymphocytes.

MATERIALS AND METHODS

BLV-INFECTED DONOR CATTLE

The number of lymphocytes from BLV-infected donors necessary to cause infection in adult recipients and the effect of natural vs experimental infection of the donor, duration of infection and age on infectivity was studied using 13 BLV-infected cattle (Table I). The 13 cattle included ten Holstein, two Holstein crosses and one Ayrshire. Six were naturally infected with BLV and seven were experimentally infected. Persistent lymphocytosis was determined by three monthly blood examinations using the Bendixen key (19). Persistent lymphocytosis was present in five animals; one animal with lymphocytosis on two examinations was placed in the suspect category. Seven of the animals were infected with BLV for more than 24 months and nine cattle were more than three years of age.

TABLE I. Data on BLV-infected Donors and Incidence of Infection in Recipient Steers

Donor Animal	Breed	Sex	Donor's Initial Infection	Lymph/ μL	Duration of Infection (Mo)	Age (Yr.)	No. pos./No. inoc. No. Cells Inoculated 1x10⁴	2x10⁴	5x10⁴
1	Holstein	F	Nat	28167	> 30	10	1/1	1/1	1/1
2	Holstein	F	Exp	3119	45	4	0/1	0/1	1/1
3	Holstein	F	Nat	4355	> 30	6	0/2	ND	1/1
4	Holstein	F	Exp	4230	4	4	0/2	ND	1/1
5	Holstein	F	Exp	8305	47	8	0/1	1/1	0/1
6	Holstein	F	Exp	12002	16	4	ND	1/1	1/1
7	Holstein	M	Nat	4995	24	4	0/1	1/1	0/1
8	Ayrshire	M	Exp	9312[a]	43	4	1/1	0/1	1/1
9	Holstein	F	Nat	12953	39	4	0/2	ND	1/1
10	Holstein	F	Nat	11959	13	3	0/2	ND	0/1
11	Holstein	F	Nat	3306	16	2	0/2	ND	0/1
12	Holstein cross	M	Exp	2262	18	2	0/1	0/1	0/1
13	Holstein cross	M	Exp	4331	18	2	0/1	ND	0/1

[a]Placed in suspect category since one lymphocyte count was not greater than 5000/μL
ND = Not determined
Nat = natural
Exp = experimental

SEPARATION OF MONONUCLEAR CELLS FROM PERIPHERAL BLOOD

Blood was collected in preservative-free heparin from the 13 BLV-infected donor cattle. Two mL of blood were carefully layered on top of an equal volume of Ficoll-hypaque (density 1.077. Ficoll, Sigma Chemical Co., St. Louis, Missouri; hypaque, Winthrop Laboratories, New York) in sterile 12 x 75 mm polycarbonate tubes and centrifuged at 400 x g for 25 min at 25°C. Mononuclear cells were removed with a Pasteur pipet, washed twice in Hanks' balanced salt solution (BSS), placed in 1 mL of RPMI-1640 medium and counted on an electric cell counter. Cell numbers were adjusted in RPMI-1640 such that 1 mL contained the appropriate number of mononuclear cells for inoculation.

INFECTION OF ADULT CATTLE

In the initial experiment, 29 adult (> 1 yr age) BLV-negative steers (Angus, Hereford and Angus-Hereford crosses) were inoculated subcutaneously with 1.0 mL containing either 1 x 10^3, 2 x 10^3, 1 x 10^4 or 2 x 10^4 mononuclear cells from the BLV-infected cattle. The equivalent amount of blood represented by these inocula was calculated for each donor animal based on the absolute number of lymphocytes. Serum samples were obtained at monthly intervals for three months postinoculation and BLV infection was determined monthly by agar gel immunodiffusion using a dual antigen (p24 and gp60) preparation (20). All sera collected on the third bleeding were tested at least twice.

A second group of 26 recipients, including 20 that were negative three months after inoculation in the above experiment, were used for a second experiment. Animals were reused due to economic considerations and because cattle experimentally infected with BLV seroconvert within three months postinoculation (Schultz, unpublished observation). The animals were inoculated subcutaneously with either 1 x 10^4 or 5 x 10^4 mononuclear cells from the BLV-infected donor cattle. Serum samples were taken at two weeks postinoculation as well as monthly intervals for three months.

DETERMINATION OF FACTORS AFFECTING INFECTIVITY

All infections caused by 1 to 5 x 10^4 lymphocytes were grouped for each donor animal to determine if experi-

mental vs natural infection of the donor, PL, duration of infection or age had an effect on the ability of lymphocytes from the 13 infected animals to cause infection in recipient steers. The mean was calculated and means were subjected to analysis of variance. Statistical independence of each category (e.g. duration of infection and age) was also verified.

RESULTS

MINIMUM NUMBER OF LYMPHOCYTES (EQUIVALENT AMOUNT OF BLOOD) CAUSING INFECTION

Information on BLV-infected donor animals and infections of recipient steers following inoculation of 1 to 5 x 10^4 mononuclear cells from donors is presented in Table I. A summary of all inoculations (10^3 to 5 x 10^4) is presented in Table II.

None of the steers inoculated with 10^3, or 2 x 10^3 lymphocytes from BLV-infected cattle seroconverted (Table II). Infection occurred in two of 17 animals (11.8%) inoculated with 10^4 lymphocytes. The equivalent amount of blood represented by 10^4 lymphocytes was calculated based on the mean number of lymphocytes per μL in the blood of the animals causing infection. Two cattle with elevated lymphocyte counts were able to cause infection with 1 x 10^4 lymphocytes or the mean equivalent of 0.72 μL of blood (Table II). Four of seven (57.1%) steers became infected following the inoculation of 2 x 10^4 lymphocytes or a mean of 2.20 μL of blood from infected donors (Table II). Eight of 13 (61.5%) steers became infected following the inoculation of 5 x 10^4 lymphocytes or a mean of 7.34 μL of donor blood.

FACTORS AFFECTING INFECTIVITY OF BLV-INFECTED CATTLE

The ability of 1 to 5 x 10^4 lymphocytes to cause infection was studied in relation to the method of initial infection (natural vs experimental) of the donor and persistent lymphocytosis, duration of infection and age of donor cattle (Table III). Analysis of variance showed no difference ($p < 0.05$) in infectivity of lymphocytes from naturally vs experimentally exposed donor cattle. An average of 27.7% of steers inoculated with lymphocytes from naturally infected and 34.0% of steers inoculated with lymphocytes from experimentally infected cattle seroconverted to a BLV-positive state (Table III).

More cattle with PL, however, were able to cause infection with 1 to 2 x 10^4 lymphocytes than were cattle without PL. Four of five cattle with PL caused infection of steers with 1 to 2 x 10^4 lymphocytes, whereas only one of seven cattle without PL caused infection with that number of cells (Table I). Likewise, when 5 x 10^4 cells were inoculated, four of five cattle with PL caused BLV infection, whereas only four of seven PL-negative cattle caused infection.

Significantly greater infectivity was found with 1 to 5 x 10^4 lymphocytes from animals infected for more than 24 months vs 24 months or less (40.3% vs 20.1%, $p < 0.05$) and from animals more than three years of age vs those three years of age or less (45.2% vs 0%, $p < 0.01$) (Table II).

TABLE II. Summary of Experimental Infection of Adult Steers Following Subcutaneous Inoculation of Mononuclear Cells from BLV-infected Cattle

No. Cells	Equivalent Amt. Blood[a] (μL)	No. Pos./ No. Inoc.	% Pos.
1 x 10^3	0.19 ± 0.10[b]	0/11	0
2 x 10^3	0.28 ± 0.20[b]	0/7	0
1 x 10^4	0.72 ± 0.50	2/17	11.8
2 x 10^4	2.20 ± 0.59	4/7	57.1
5 x 10^4	7.34 ± 0.80	8/13	61.5

[a]Based on mean lymphocytes counts of cattle causing infection
[b]Based on mean lymphocytes counts of all donor cattle

TABLE III. Relationship of Source of Infection, Persistent Lymphocytosis, Duration of Infection and Age on Infectivity of Lymphocytes from BLV-infected Cattle

		Infection with 1 to 5 x 10^4 Cells	
	No. Cattle	No. Inoculated	% pos ± SE
Souce of Donor Infection			
Natural	6	18	27.7 ± 4.9
Experimental	7	19	34.4 ± 2.7
Lymphocytosis			
Positive	5	15	44.3 ± 4.9
Negative	7	18	20.1 ± 2.7
Suspect	1	4	50.0
Duration of Infection			
> 24 mo	7	20	40.3 ± 2.7[a]
≤ 24 mo	6	17	20.9 ± 4.9
Age			
> 3 yr	9	28	45.2 ± 4.0[b]
≤ 3 yr	4	9	00.0

[a]$p = < 0.05$
[b]$p = < 0.01$

DISCUSSION

The present study was undertaken, to determine the number of transferred lymphocytes required to cause infection in adult cattle, and to assess factors which may affect the infectivity of BLV infected cattle. Adult cattle were used rather than calves since infection with BLV occurs most frequently in cattle greater than one year of age (14,15,21).

Transmission of BLV was not accomplished by subcutaneous inoculation of 10^3 or 2 x 10^3 lymphocytes; however, two animals were infected following the inoculation of 10^4 lymphocytes. This inoculum was the equivalent of 0.36 μL blood from one donor and 1.07 μL of blood from the second donor. Doubling the number of lymphocytes inoculated from 10^4 to 2 x 10^4 increased the percentage of positive recipients by almost fivefold (from 12% to 52%), whereas increasing the number from 2 x 10^4 to 5 x 10^4 only increased the

number of seroconversions by a factor of 1.08 (from 57% to 62%). These data suggest that the commonly cited finding that 2500 lymphocytes from a BLV-infected animal caused infection in a calf (18) should not be taken as a rule for BLV transmission, since 2500 lymphocytes represents an exceptionally low dose of cells.

Factors such as age (14,15,21) and genetic predisposition (22) have been studied with regard to susceptibility to BLV-infection; however, no studies have been reported on factors affecting the infectivity of cattle with BLV. Results from the present study suggest naturally vs experimentally acquired infection has no effect on the ability of 1 to 5 x 10^4 lymphocytes to cause infection in adult cattle. Results of this study also suggest that cattle with PL were more infectious than those without PL when 1 to 2 x 10^4 lymphocytes were transferred; however, both may be equally infectious when 5 x 10^4 lymphocytes are transferred. These data indicate that 5 x 10^4 lymphocytes represent the lowest number of cells from a hematologically normal donor able to cause infection, whereas the lowest infecting dose from animals with PL is 10^4 lymphocytes.

The above data represent comparisons of cattle with and without PL on a per lymphocyte basis, suggesting that more of the lymphocytes from animals with PL are infected with BLV than are lymphocytes from cattle without PL. Considering this to be true, together with the fact that lymphocyte numbers in PL increase to two to ten times their normal number, cattle with PL must be considered more infectious than cattle which are hematologically normal.

Bovine leukosis virus is not produced by all peripheral blood lymphocytes. This is evidenced by Kenyon and Piper's (23,24) description of two subpopulations of B cells in cattle infected with BLV. One subpopulation produced BLV as demonstrated by its ability to induce syncytia in indicator cells; the other spontaneously incorporated tritiated thymidine. Although the two subpopulations were found in both PL and non-PL infected cattle, data were not presented on differences, if any, in B cell subpopulation sizes among cattle with and without PL.

The greater infectivity of lymphocytes from BLV-infected animals with PL than those without PL may be due to (a) a greater percentage of cells that produce BLV and/or (b) greater virus production by cells from cattle with PL. Greater BLV production may depend on the relative number of provirus integration sites in PL and non-PL animals, assuming that mature viruses could arise from each integration site.

Both the duration of infection and the age of infected animals had a significant effect on infectivity. These two categories were found to be statistically independent, in that not all animals older than three years had been infected for more than 24 months. The four animals less than three years of age, however, had also been infected for less than 24 months. The inability of lymphocytes from these four animals to cause infection in recipient cattle may have been due either to age or duration of infection, or both. The lack of PL in three of the four probably played less of a role than age or duration of infection since four of seven animals without PL were able to infect cattle when 5 x 10^4 lymphocytes were inoculated. An increase in the number of virus-infected cells over time may account for heightened infectivity of older cattle.

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BOOK REVIEW

ADVANCES IN VETERINARY SCIENCE AND COMPARATIVE MEDICINE. VOLUME 27. *Edited by C.E. Cornelius and C.F. Simpson. Published by Academic Press, Don Mills, Ontario. 1983. 519 pages. Price $78.50.*

This volume well indicates the breadth of veterinary medicine and as stated in the original volume attempts to "maintain a broad but fluid frontline of modern knowledge".

M.J. Newman and D.F. Antczak have provided a well referenced review on the recent impressive research pertaining to histocompatibility polymorphism of domestic animals. Another immunity related topic is the immune mediated diseases covered by W.J. Dodds. The latter topic is primarily devoted to blood problems in the dog and the socalled autoimmune diseases that affect the red blood cell and platelet. Blood constituents are also covered in deer, specifically a review of sickled red cells in Cervidae by W.J. Taylor.

Malformation problems are covered in three chapters. J.H. Rowland and A.G. Hendrickx have reviewed corticosteroid induced teratogenicity in laboratory animals with particular emphasis on corticosteroid mediated cleft palate. After a brief overview of the nature, frequency and causes of congenital defects in the bovine, H.W. Liepold, K. Huston and S.M. Dennis provide a very useful list, with brief descriptions, based on the different body systems. Avian lymphoproliferative diseases are the most commonly occurring neoplasms in poultry. K. Perk has provided an update on the classification, pathological features and viral causes of these leukemia-like diseases.

Two chapters pertaining to ticks are included in this volume. An interesting discussion of ascaricide resistance in ticks by K.R. Solomon and the provision by G. Uilenberg on the current status of heartwater in cattle. In the latter chapter transmission, diagnosis and prevention are given careful attention.

The increased spread of the virus causing Rift Valley Fever is of concern in view of its transmission to human subjects. A. Shimahony and R. Barzilai have given an extensively reviewed summary of this condition, the viral agent and the various vaccines utilized for control. H.P. Riemann and B. Abbas present a summary of the diagnosis and control of bovine paratuberculosis. They have attempted to indicate the efforts being made to improve the accuracy of diagnostic procedures and the progress made in the control and eradication of Johne's disease.

An interesting paper which will provide a good introduction to the subject is the control and therapy of fish diseases by J.B. Gratzek. The introduction of courses on fish diseases into the veterinary curriculum indicates the increasing importance of this topic. Another topic receiving increased attention is embryo transfer in domestic animals. G.B. Anderson has given a description of the present technology in this area, particularly in cattle where the major effort has been devoted.

Different chapters in this book will appeal to different readers. However, no institution that serves the interests of veterinary medicine in its broadest sense can afford to be without this latest volume in a respected and well regarded series. — *R.M. Liptrap.*